Chapter 6

Contribution of Autophagy to the Physiological and Pathophysiological Functions in the Mammalian Testis

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Additional information is available at the end of the chapter

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Abstract

Mammalian spermatogenesis is a high regulated biological process occurring in the seminiferous tubules in the testis. The processing of this program requires delicate balance between cell proliferation, differentiation, apoptosis, and expedite cell interaction. Autophagy, an evolutionarily conserved cell reprograming machinery, had been shown to function as an important regulatory mechanism in spermatogenesis and steroid production in testis. Herein, we mainly focused on our understanding of autophagy in mammalian testis. By showing autophagy in physiological and pathophysiological conditions, we try to elicit the regulatory role of autophagy in spermatogenic cells and somatic cells of testis. Moreover, this review is intended to point out factors and mechanisms, which contribute to the initiation of autophagy in testicular cells.

Keywords: autophagy, physiological functions, pathophysiological functions, spermatogenesis, testis

1. Introduction

Macroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved mechanism of sequestering part of cell component into cyclic processes to reverse adverse microenvironmental conditions, including limited nutrient supplies, hypoxia and some other stresses. By autophagy, misfolded proteins and impaired organelles are packaged by double membrane structure and delivered into lysosomes for cargo degrading. The basic structure of autophagosome was first revealed by Ashford and Porter [1], who described membrane-bound vacuoles in rat liver cells. As research continues, researchers observed autophagosomes in many other cell types, suggesting that autophagy is a ubiquitous mechanism in

eukaryotes. Of note, autophagy is known as the only mechanism to degrade large structures, including organelles and misfolded proteins. Phylogenetically conserved role of autophagy is considered to balance the metabolic homeostasis of cell under dwindling nutrient supplies and other external perturbations. Cellular autophagic machinery induces rapid mobilization of endogenous dispensable reserves, which ensures the speedy generation of retrieving fuel for ATP synthesis [2]. Therefore, the initiation of autophagy reduces the sensitivity of cell to nutrient deprivation. In tumor cell lines, autophagy, as a mean to anti adverse microenvironment, is more prevalent than normal cells. Also, autophagy is indispensable to the survival of normal cells, and the maintenance of basal autophagy is crucial to the survival and function of many cell types in physiological settings, especially in nerve cells [3]. The abnormity of autophagy is related to many diseases such as aging, cancer, cardiac disease, and obesity.

In addition, various internal disorders and external stresses might function as initiators of autophagy such as DNA injury, toxicant exposure, heat stress, hypoxia and nutrient deprivation. Autophagy can protect the survival of cell or accelerate the process of cell apoptosis depending on the situation of cellular microenvironment. The relationship between autophagy and apoptosis has been detailedly documented in many papers [4]. However, the participation of autophagy in testicular function has received little attention in the literature. Here, we will address the consequences for testicular endocrine homeostasis and spermatogenesis in physiological and pathophysiological conditions, thereby eliciting the regulatory roles of autophagy on spermatogenic cells and somatic cells in testis.

2. The involvement of autophagy in spermatogenesis

The main testicular functions are related to endocrine secretion and the output of functional sperm. The secretion of endocrine is mainly accomplished by Leydig cells, whereas the production of mature functional sperm is initiated from so-called seminiferous tubules. During spermatogenesis, the germ cells undergo several structural reorganizations including the generation of the acrosome, the condensation of the nuclear chromatin, the rearrangement of the mitochondria, the assembly of the sperm flagella, and the removal of unnecessary cytoplasm to product functional sperm [5]. The development and differentiation of germ cells require drastic cytoskeleton remodeling, enhanced energy consuming and components degrading. For this, autophagy can "kill two birds with one stone" by eliminates needless cellular materials and providing supports for the subsequent creation of new components [6]. It had been established that autophagy is basically induced in diploid germ cells, while deeply involved in the restructure of spermatid shaping during spermiogenesis. The functional role of autophagy had demonstrated to be an indispensable mechanism for sperm production, and the deficiency of autophagy finally results in male infertility.

2.1. Autophagy in diploid germ cells

Spermatogenesis is the process that transforms spermatogonia into sperm over an extended period of time takes place in seminiferous tubule boundaries of the testis. Mammalian testicular diploid germ cells, including spermatogonia and spermatocyte, proliferate first by repeated

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mitotic divisions and then by meiosis to form haploid spermatids. During this period, chromosomes duplication, genetic recombination, and many other cellular meiotic accessary processes occurred ensuring that high differentiated sperm could be successfully released into the tubule lumen [7]. The activation of autophagy is crucial for the maintenance of cellular energetic balance in spermatogenic cells. In testis, spermatogonial stem cells (SSCs) are suggested as the foundation of mammalian spermatogenesis and fertility. Histologically, SSCs are rare, contributing only 0.03% of all germ cells in rodent testis [8]. Similar with the property of hematopoietic stem cells (HSCs), SSCs sustain life-long spermatogenic property. Studies had suggested that the occurrence of autophagy is essential for HSCs maintenance, and loss of autophagy lead to accumulation of mitochondria, reactive oxygen species (ROS), and DNA damage [9]. However, there are no investigations about the regulatory role of autophagy in spermatogonia. It is possible that the maintenance of cellular basal autophagy in SSCs is also a self-protective mechanism during its differentiation and self-renewing similar with that of HSCs. Noteworthy, the autophagy level of SSCs is relatively lower than other types of germ cells in testis, such as round and elongating spermatids under physiological conditions. Furthermore, the autophagy is not involved in the postnatal development of spermatogonium and spermatocyte because the absence of autophagy at spermatogonium (d7) and spermatocyte (d15) stages [10].

Under physiological conditions, high rate of cell division during spermatogenesis implies correspondingly elevated levels of mitochondrial oxygen consumption and ROS generation in spermatogonia. Mechanistically, the production of ROS is a required physiological event for the renewal of spermatogonia, the functional maturation and capacitation of spermatozoa [11]. On the other hand, ROS also participates in the induction of cellular autophagy via initiating diverse downstream signaling pathways [12, 13]. Glutathione (GSH) plays a key role in the antioxidant defense of spermatogonial cells, and high concentration of GSH has been reported in mouse testicular germ cells [14]. It had been established that GSH is involved in the regulation of autophagy in many types of cells [15, 16]. In spermatogonia, the depletion of GSH leads to the induction of autophagy. Interestingly, the depletion of GSH does not influence the level of ROS, while contributing to the downregulation of S-glutathionylated proteins, protein S-glutathionylation is initially described as a protein oxidation process, thereby leading to the induction of autophagy in spermatogonia [17]. These evidences suggest that the oxidative stress might be one of the main factors that physiologically turn on autophagy in SSCs by controlling the level of S-glutathionylated proteins in spermatogonia. Mechanistic studies suggest that GSH depletion initiates autophagy by an AMPK independent signaling pathway. The activation of autophagy induced by GSH depletion does not contribute to the alteration of energetic status in spermatogonia. It is possible that physiological fluctuation of autophagy level around the basal autophagic thresholds is accepted by germ cells.

During the initiation of autophagy, Beclin1 and p62 are two important proteins that ubiquitously implicated in the formation of autophagosomes and the recognition of autophagic cargos in many cell types. The expression of Beclin1 frequently shows similar tendency with that of LC3-II, while p62 shows opposite tendency. And, it is accepted that the expression of Beclin1 and p62 is not always consistent with expected tendency during the initiation of autophagy. However, there is a controversy about the expression of Beclin1 in spermatogonia under nutrient deprivation. As Mancilla et al. suggest that the expression of Beclin1 is

not altered in nutrient starvation induced autophagy, while Wang et al. draw an opposite conclusion [18]. It is possible that inconsistent results are caused by their different starvation conditions. The activation of NF-κB pathway is recognized as the mechanism of autophagy induction in spermatogonia under nutrient deprivation. In addition, ankyrin repeat domain 49 (ANKRD49), an evolutionarily conserved protein highly expressed in testes, can significantly enhance the transcriptional activation of NF-κB, therefore upregulating autophagy level of spermatogonia under starvation [18].

Apoptosis, necrosis, and autophagy have been described as typical cell death programs. In the testis, the death of spermatocyte is crucial for controlling sperm output [19]. With this regard, apoptosis, necrosis, and autophagy are mechanistically related machineries in spermatocyte for the control of testicular homeostasis. Under physiological conditions, the death of spermatocyte is associated with available energy supply. It has been documented that spermatocytes use lactate rather than glucose as their primary substrate for the production of ATP [20]. In vitro studies revealed that after 6 h of culture a significant increase of cell death is detected for spermatocytes cultured in glucose, while there is no significant increase in cells cultured with lactate. However, autophagy levels are significantly increased in spermatocytes cultured with glucose or lactate after 12 and 24 h, suggesting that autophagy might function as a pro-death role in rat spermatocytes under certain metabolic conditions [21]. In spermatocyte, the molecular mechanism is not yet clear about what factors determine whether autophagy acts as a cytoprotective defender or a cytotoxic trigger and whether cytotoxicity occurs as the result of self-cannibalism, the specific degradation of cytoprotective factors, or other as of yet undefined mechanisms [22]. It is certain that autophagy is not involved in the regulation of spermatocyte in newly born infant, because the expression of autophagic marker proteins is absent from spermatocytes at postnatal day 15 [23]. However, the physiological roles of autophagy in spermatocyte of adult mice still remain unknown.

Relatively, the autophagy levels of both spermatogonia and spermatocyte are maintained at a low level compared with other types of germ cell in mammalian testis under physiological conditions. It is established that the autophagy level is high related to inner cell status and outer microenvironments. In testis, the nutrition status of germ cells is directly associated with their mother cells, Sertoli cells. And, except for the early phase of spermatogenesis from type B spermatogonia up to preleptotene and leptotene spermatocytes, the entire process of germ cell development is isolated from the systemic circulation because of the blood-testis barrier (BTB) created by tight junctions (TJ) between Sertoli cells near the basal lamina [24, 25]. Thus, it is clear that spermatogonia and spermatocytes are suffered to different physiological environments. The difference of physiological hormonal subjection might be one of the main factors that led to the inhibition of autophagy in diploid germ cells. In testis, follicle-stimulating hormone (FSH) in all cycles is to increase spermatogonia and subsequent spermatocyte numbers, which is similar to the physiological role of FSH on granulosal cells. Previous studies had demonstrated that FSH functions as an autophagy inhibitor in ovarian granulosal cells [26]. We hypothesis that FSH might also contribute to the inhibition of autophagy in testicular diploid germ cells, while detailed molecular pathways in diploid germ cells still remain to be documented.

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2.2. The role of autophagy and spermatid differentiation

Spermiogenesis is a sophisticated and highly ordered spermatid differentiation process that requires reorganization of cellular structures and readjustment of cellular physiological functions. The successful removal of cytoplasm is thought to be critical for the generation of functional and motile spermatozoa. The dysfunction of spermatozoa is mainly caused by the abnormal of spermatozoa head or the coil of its flagellum. Autophagy is deeply involved in the processes of spermatozoa formation, and the deficiency of autophagy leads to various spermatozoa defects, which could be classified into three groups, the abnormal of spermatozoa head, the coil of spermatozoa tail, and the aggregate of spermatozoa [23]. For the spermatozoa with bent head, a large portion of cytoplasm is remained connecting the bent head and the tail, thus lead to the inhibition of its beating. For the spermatozoa with coiled tail, the sperm tail is seriously coiled with mislocalized and poorly condensed mitochondria, while aggregated spermatozoa is characterized by the presence of clustered sperm tails and is wrapped by membrane and some cytoplasm.

In spermatozoa, the acrosome is a specialized membranous organelle located over the anterior part of the sperm nucleus, which is important for the dispersion of cumulus cells and the penetration of the zona pellucida of the oocyte during fertilization. The formation of acrosome involves the reprogramming of cellular cytoskeleton, which requiring the induction of autophagy to assist the rearrangement of cellular cytoskeleton. Indeed, comparing with diploid germ cells, the expression of autophagy-related proteins such as LC3 and Atg7 are significantly higher in elongated spermatid. Furthermore, the expression of LC3 could be observed firstly in round spermatid (d20) of postnatal testis, which indicating the involvement of autophagy in early testicular spermatid development. Ablation of autophagy by germ cell-specific knockout of Atg7 leads to the decrease of testicular weight, the detachment of premature germ cells, and the malformation of spermatozoa, which significantly reduce the fertility of male mice [10]. Particularly, many spermatozoa from Atg7−/− mice are endowed with irregularly shaped round heads similar to human globozoospermia, a severe fertility disorder characterized by round-headed spermatozoa with malformed acrosome or without acrosome at all. In addition, the deficiency of Atg7 also leads to many other acrosomal defects, such as the mis-localization, the deformation and the fragmentation of spermatozoal acrosome; thus, they failed to acquire the typical crescent moon shape [10].

The formation of acrosome is grouped into four phases: Golgi, Cap, Acrosome, and Maturation [27]. Autophagy participates in acrosome biogenesis starting in the Golgi phase. In normal conditions, Golgi apparatus-derived proacrosomal vesicles are fused into a single acrosomal vesicle attached to one end of the nucleus in the Golgi-phase spermatids. After Atg7 disruption, multiple small vesicles of the Golgi-phase spermatids are failed to fuse with each other, thereby showing multiple acrosomal structures. In cap phase, 10% of the spermatids had multi acrosomal vesicles or aggregates, and the accumulation of proacrosomal vesicles derived from the Golgi apparatus leads to the shrinkage of acrosome [10]. These evidences suggest that the malformation of acrosomes most likely caused by the failure of proacrosomal vesicles to fuse and be transported to the preacrosome at one end of the nuclei. Mechanistically, the function of Atg7 in acrosome biogenesis might be similar to its role in

autophagy induction. Within autophagy, LC3-lipid conjugation is a reversible process, LC3 residing on the outer face of the vesicle can be recycled by Atg4, whereas LC3 on the inner surface is ultimately degraded. In spermatid, LC3 is only colocalized with the trans-Golgi network marker TGN38 rather than the acrosome maker sp56. Therefore, membrane-associated LC3 might participate in the fusion of Golgi apparatus-derived proacrosomal vesicles and their transportation to the acrosome. After fusion with the acrosome, LC3 will be either recycled or degraded. After Atg7 disruption, LC3 is failed to colocalize with TGN38, causing the accumulation of proacrosomal vesicles in the concave region near the trans-Golgi stacks [10]. Finally, this accumulation impairs the increase in acrosome volume in the later stages, whereupon resulting in defective acrosome formation.

A mammalian spermatozoon is characterized by two morphological and functional components; the head and the flagellum, both parts are optimized for special tasks. The formation of spermatozoa head and flagellum requires the mobilization and specialization of cytoskeleton in spermatid. During this process, autophagy is extensively involved in the regulation and the remolding of cell shapes by altering cellular cytoskeletons [28]. In round and elongating spermatids, autophagy is recognized as a potent regulator of cell structures in both types of germ cells. It has been established that except for the role of autophagy on acrosome shaping, it is also involved in the formation of sperm flagella via the rearrangement of the mitochondria and the elimination of unnecessary cytoplasm to facilitate spermatozoa motility. Gene knockout corroborated the role of autophagy in spermiogenesis. As mice spermatids begin to elongate from step 8, the deficiency of Atg7 makes no change on spermatids before step 8 [10].

PDLIM1 (PDZ and LIM domain protein 1) is a member of the PDZ and LIM protein family, containing an N-terminal PDZ domain and a C-terminal LIM domain. It is acknowledged that PDLIM1 acts as a scaffold to bring other proteins to the cytoskeleton and is also involved in cytoskeleton reorganization in many types of cells [29, 30]. During spermiogenesis, PDLIM1 functions as a mediator between autophagy and cytoskeleton organization. Under normal conditions, the degradation of PDLIM1 by the autophagy-lysosome pathway is needed to maintain a proper dynamics of the cytoskeleton network whereupon assuring that spermatids differentiation could be processed smoothly. The disruption of autophagy results in failure engulf of PDLIM1 by autophagosomes, thereby leading to their accumulation in the cytoplasm. The accumulation of PDLIM1 disrupts the proper dynamics of the cytoskeleton and finally leads to the inefficient cytoplasm removal during spermiogenesis. In normal testis, F-actin signal is stronger than PDLIM1 in the elongating spermatids, and also, F-actin based acroplaxome provides a docking site for the acrosome development, thus anchors it to the spermatid nucleus [31]. Autophagy impairment strongly increases the level of PDLIM1 in spermatids and disrupts the organization of cytoskeleton, thus leading to the disorganization of flagellar “9+2” structure and other cytoskeletal components in spermatozoa [23]. The well organization of spermatozoal flagellum is crucial to the normal motility of spermatozoa, while the deficiency of autophagy significantly changed sperm motility parameters including the average path velocity (VAP), straight-line velocity (VSL), and curvilinear velocity (VCL).

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In round spermatids, autophagy is also involved in the degradation of other cellular components. Among which, the degradation of chromatoid body has been recently uncovered. In germ cells, the chromatoid body (CB) is an unusually large germ granule, which is initially formed in the cytoplasm of late pachytene spermatocytes. After meiosis, CB is condensed to its final form and maintain a distinct cytoplasmic feature throughout the differentiation of round spermatids [32]. During the elongation of spermatids, the size of CB will be shrink forming a ring around the base of the flagellum [33]. The accessary material from the CB is finally discarded together with the rest of the cytoplasm in the residual body. It is demonstrated that the clearance of these materials is mainly undertook by autophagy via an FYCO1-dependent pathway, and the induction of autophagy enables the homeostasis of CB [34]. Mechanistically, FYCO1 functions as a docking site for LC3 and LAMP1-positive membranes mediating the recruitment of autophagosome and lysosome to the CB. In addition, an intriguing option is that some specific RNA species are also eliminated via FYCO1-mediated autophagy [35]. However, the disruption of FYCO1 pathway in round spermatid somewhat does not impair the fertility of male.

3. Autophagy and the functions of testicular somatic cells

Leydig cells and Sertoli cells are two types of somatic cells exist in mammalian testis. Histologically, Leydig cell and Sertoli cells share different locations in testis, which implies diverse hormonal exposure and different physiological functions. Both types of testicular somatic cells adopt autophagy as a regulatory mechanism for the maintenance of cellular homeostasis. During spermatogenesis, physiological orders are assigned to each seminiferous tubule, and seminiferous tubule substantially acts as a functional unit of testis. Leydig cells are histologically localized in the interval of seminiferous tubules, while it also exerted in the regulation of spermatogenesis by secreting testosterone. In addition to the role of testosterone on germ cells, some of the cellular biological programs are also selectively regulated by testosterone via an autophagy-dependent pathway in Sertoli cells. Comparatively, the Sertoli cells maintain tight contact with germ cells in seminiferous tubules. In essence, the functions of Sertoli cells are related to its paralleled structural basis, the malformation of cellular structure frequently leads to the infertility of male. Autophagy plays pivotal roles in the regulation of Sertoli cell functions via cytoskeleton adjustment [36].

3.1. Autophagy and Leydig cell function

Previous researchers had revealed the regulatory roles of autophagy in steroid production and secretion [37]. In male mammal, testis contributes about 95% of total circulating testosterone, and Leydig cell is the primary testosterone contributor in mammalian testis. Testosterone is necessary for male fetal sexual differentiation, adult secondary sex characteristics maintenance, and spermatogenesis. Like many other types of steroid-producing cells, Leydig cells are typically own enlarged mitochondria than other cell types. The production of steroid is an energy-intensive engineering, which directly related to the damage of mitochondrial function. The involvement of autophagy in cellular organelle degradation had been reported in other

cell types [38]. It has demonstrated that the relative frequency of autophagy in Leydig cells is higher than many other cell types [39]. Consistently, abundant autophagosome engulfed organelles are also observed in rat Leydig cells, most of the organelles enclosed in the autophagic vacuoles are SER and mitochondria, organelles that involved in the production of androgens. These evidences lead to the hypothesis that the autophagic activity might relate to the regulation of hormonal secretion in Leydig cells. Indeed, the process of autophagy is high related to the production of testosterone in rat Leydig cells, and the deficiency of autophagy is frequently associated with the dysregulation of testicular homeostasis [40]. However, little is known about the relationship between autophagy and testosterone production in Leydig cells under physiological conditions.

It is acknowledged that autophagy is a predominant cytoprotective rather than a self-destructive process in normal cells [41]. Accordingly, autophagy is involved in mediating protective effects in multiple rodent models of organ damage affecting the heart, liver, nervous system, and kidney. Reduced autophagy level has been associated with accelerated aging process, while promote autophagy could partially protect cell from natural aging process [42]. The induction of autophagy is also involved in the maintenance of testosterone level in rat testis. In the old rat, the accumulation of ROS is significantly increased comparing with that of young rat. ROS act as one of the main factors that lead to the downregulation of StAR (steroidogenic acute regulatory protein) protein level and the secretion of testosterone by activating p38 mitogen-activated protein kinase or c-Jun [43]. In Leydig cells, autophagy regulates the accumulation of ROS by promoting the clearance of damaged mitochondria, oxidized cellular substrates, and by activating antioxidant systems. Inhibition of autophagy by disrupting Beclin1 decreases the expression of StAR, while inducing autophagy in Leydig cells from aged or young rat by rapamycin increases the expression of StAR under the stimulation of LH [40]. Thus, it is possible that the downregulation of testosterone in aged Leydig cells might result from two aspects: (1) the deficiency of autophagic machinery and (2) the increase of ROS level. However, the increase in ROS could also be attributed to the compromise of autophagic efficiency.

Endocytosis is an active transport machinery, by which a cell transports molecules and proteins into the cell by engulfing them in an energy-using process [44]. Similar with the function of autophagy, endocytic mechanism plays an important role in regulating how cells interact with their environments. Both endocytosis and autophagy are the major pathways for transporting materials to lysosomes in animal cells, the former being responsible for uptake of extracellular constituents while the latter for degradation of cytoplasmic components. In the Leydig cells, the endocytosis remains close cooperation with autophagy whereupon leading to the degradation of respective contents. It had been shown that late endosomes deliver their endocytosed contents and lysosomal enzymes to the early autophagosomes, implying that the endocytosis and autophagy are seamlessly connected in rat Leydig cells. The collaboration of both mechanisms is efficiently mobilized in Leydig cells under physiological conditions. Different with other cell types, rat Leydig cells morphologically show typical autophagy-related morphologies, as the early signs of autophagy, called preautophagosomes, can be easily observed in the ultrastructure of Leydig cells, while these structures are rarely shown in other normal cell types even when autophagy is induced [45].

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3.2. Autophagy is required for structural modulation in Sertoli cell

During spermiogenesis, the differentiating germ cells undergo a successive morphological transformation from round spermatids to sperm, which requires cellular remodeling of spermatids and the assistance of Sertoli cells. The Sertoli cell is involved in the degradation of many useless components within seminiferous tubule, such as spermatid residual bodies (RB) and the apoptotic germ cells. Sertoli cell has a prominent ability to metabolize those phagocytized materials. In testis, the homeostatic phagocytosis of Sertoli cells varies depending on seminiferous epithelium cycle and reaching its maximum during spermiation [46]. In Sertoli cells, both autophagy and phagocytosis may undertake similar mission under certain circumstance, especially once a phagocytic vesicle has entered a cell. Numerous evidences had suggested that autophagy is associated with the process of phagocytosis and is atypically implicated in the degradation of external substrates entering via phagocytosis in Sertoli cells [47]. Generally, blood-separated tissues use their tissue specific nonprofessional phagocytes for homeostatic phagocytosis [48]. In the testis, Sertoli cells manage illegitimate substrates and legitimate substrates with different pathways. Exposing of cultured Sertoli cells to either illegitimate (such as photoreceptor outer segments generated from other tissue) or legitimate substrates (such as residue body generated by differentiating germ cell), both substrate types are ingested by phagocytosis. Nevertheless, autophagy is selectively involved in the degradation of those illegitimate substrates in Sertoli cells, and the inhibition of autophagy significantly retarded the degradation of illegitimate substrates [47].

Sertoli cells play pivotal roles in the regulation of spermatogenesis by providing structural support and nourishment to developing germ cells, controlling the self-renewal and differentiation of spermatogonial stem cells (SSCs), protecting the autoreactive immune response of germ cells, and releasing spermatids at spermiation [49]. In the seminiferous epithelium, functional cell interconnections are maintained by Sertoli-Sertoli cell and Sertoli-germ cell junctions [50]. The Sertoli cell ectoplasmic specialization (ES) and the spermatid-containing acrosome-acroplaxome-manchette complex are two cytoskeletal structures that play important roles in the shaping of sperm head [51]. Among which, the ectoplasmic specialization (ES) is composed by two components, an actin-based atypical adherens junction between adjacent Sertoli cells at the blood-testis barrier (BTB) termed as basal ES, whereas between Sertoli cells and spermatids near the luminal surface of the tubule termed as apical ES [52]. The basal ES function as an important component of the blood-testis barrier (BTB) [53, 54] and apical ES (aES) interacts with the acrosome of the elongating spermatid and mechanically assists the shaping of spermatid head [50]. The function of apical ES is also related to the movement of spermatid cell and the release of the matured spermatozoa during spermiation. Successively, transportation of developing germ cells across the seminiferous epithelium is important for the processes of spermatogenesis, which requires the dynamic restructuring of ES in the epithelial cycle [23, 55, 56]. It has been demonstrated that autophagy is involved in the regulation of ES. The deficiency of autophagy by Atg7 or Atg5 ablation disturbs the assembly of both apical ES and basal

ES in the seminiferous epithelium. Mechanistic studies revealed that similar with that of spermatid, autophagy disruption impairs the degradation of PDLIM1 thereby resulting in its accumulation in Sertoli cells. Therefore, the organization of cytoskeleton in Sertoli cells is perturbed. PDLIM1 might be the primary substrate of the autophagy to regulate cytoskeleton organization, because the proper organization of the cytoskeletal structure could be significantly restored by knockdown of Pdlim1 gene in autophagy-deficient Sertoli cells. Successful organization of cytoskeleton in Sertoli cell is highly related to the produce of functional spermatozoa. The accumulation of PDLIM1 disrupts the F-actin hoops of the apical ES and related microtubule-based structures in the seminiferous epithelium, which ultimately leading to the disruption of Sertoli cell-germ cell communication thereafter contributing to the malformation of sperm head [36].

However, although autophagy plays pivotal roles in apical ES formation as well as basal ES assembling, it is not exert in the assembly of tubular and bulbous structures of TBCs (tubulobulbar complexes). In Sertoli cells, TBCs are located on both Sertoli-Sertoli cells and Sertoli cell-spermatids interface and are implicated in the restructure of ES, excess spermatid cytoplasm removing and spermatid acrosome shaping [57]. The cytoskeletal remolding of TBCs is also important to the release of sperm and the translocation of spermatocytes. Unwanted distribution of TBCs directly impaired the function of Sertoli cells as well as the communication between Sertoli cells and germ cells. Liu et al. suggested that the different influences of autophagy on apical ES and TBCs assembly might come from their different F-actin arrangements, because F-actin is packed in hexagonal arrays in the ES, while it appears as an embranchment surrounding the tubular portion of TBCs [36, 58, 59]. Furthermore, the incorrect distribution of TBCs might also be resulted from the abnormal structure of apical ES or the malformation of sperm head, whereas these possibilities still remain to be uncovered by further experimental data. Interestingly, the marker proteins that implicated in SSCs self-renewal or meiosis show no changes after the disruption of autophagy in Sertoli cells, implying that autophagy is dispensable in the self-renewing of SSCs and meiosis process of germ cells.

Androgen-binding protein (ABP) is a kind of sex hormone-binding globulin (SHBG) produced by testicular Sertoli cells, which specifically binds to and reduce the lipotropism of testosterone or dihydrotestosterone, making them more concentrated in the seminiferous tubules. High concentration of ABP is required for the process of spermatogenesis in the seminiferous tubules and the maturation of sperm maturation in the epididymis. In mammal, the production and the secretion of ABP are regulated by FSH, oestradiol, and testosterone [60–62]. In Sertoli cells, testosterone participates in the synthesis and the secretion of ABP by autophagy. In vitro studies suggest that ABP is colocalized with LC3 in primary rat Sertoli cells, and inhibition, or stimulation of autophagy considerably change both the expression pattern and level of ABP in Sertoli cells without affecting the expression of ABP mRNA, implying that the regulatory role of autophagy on the degradation of ABP is only works on its protein level. Furthermore, the inhibitory function of testosterone on autophagy is also influenced by testosterone concentrations, as enhanced concentration of testosterone further inhibits autophagic pathway [63]. Importantly, although hypoxia exposure further enhances the autophagy level of Sertoli cells, but hypoxia-induced autophagy does not change the expression of ABP in

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rat primary Sertoli cells, suggesting that the degradation of ABP is independent of hypoxia-induced autophagy.

4. Autophagy and testicular toxicology

The process of spermatogenesis requires well-balanced germ cell proliferation, differentiation, and death in the testis [64, 65]. However, this process can be disturbed by several endogenous or exogenous factors, including withdraw of gonadotropin or testosterone, chemical insults, heat stress, and radiation exposure. Cell apoptosis and autophagy are two major morphologically distinctive forms of programmed cell death (PCD) that play crucial roles in the development and the control of male reproductive functions. The crosstalk between autophagy and apoptosis is sophisticated in the sense that they might act synergistically or antagonistically with each other in the process of cell life and death. The normal operation of autphagic process is related to many physiological functions, whereas the dysfunction of autophagy leads to numerous diseases in human. In testis, evidences have demonstrated that autophagy plays important roles in testicular pathologies caused by oxidative stress, heat stress, toxicant exposure, and radiation exposure.

4.1. Autophagy and testicular homeostasis

Ample of evidences has documented the correlation between toxicant exposure and germ cell death. It is well known that cell may activate self-protective mechanisms in response to exogenous insults, such as chemical exposure. The activation of autophagy is important to the maintenance of cellular functions and may partially rescue the dysfunction of tissue homeostasis under adverse environments [66]. Chemical exposure is high related to the out control of tissue homeostasis. Exposure of testis to toxicants frequently leads to the activation of autophagy by different signaling pathways. Exposing testis to BPA (Bisphenol A) leads to the activation of oxidative stress, which activates autophagy mainly by inhibiting mTOR signaling pathway. Meanwhile, the phosphorylation of AMPKa and the expression of p53 might act as a contributor to the upregulation of autophagy under BPA exposure. Of note, the expression of Beclin1 is not upregulated accompany by autophagy in testis under BPA exposure [67]. With NaF exposure, the autophagy is abnormally increased as evidenced by the synchronized increase of p62, suggesting that NaF exposure impairs autophagic machinery and result in the accumulation of autophagosomes in testis [68]. In addition, autophagy is also involved in the regulation of testicular homeostasis under other toxicant treatments, for example, see Ref. [69]. As testis is composed by germ cells and somatic cells, many studies specifically evaluated the involvement of autophagy in germ cells or somatic cell to elucidate the mechanisms and functions of autophagy under specific conditions.

4.2. Autophagy and germ cell injuries

Autophagy is referred to as programmed cell death type 2, the process of which might be excessively induced under stresses, and the abnormal induction of autophagy is high related

to cellular apoptosis especially under severe adverse conditions. It has been established that testicular heating can disturb spermatogenesis and cause subfertility, some testis-related diseases including cryptorchidism are also linked to testicular heating, in which the testis is exposed to body temperature rather than scrotal temperature, whereupon lead to abnormal testis function and damaged spermatogenesis in these settings [70, 71]. Autophagy is recognized as one of the regulatory factors that participate in the death of heat-treated somatic cells [72]. Similarly, heat treatment on mouse testis could also upregulate the induction of apoptosis and autophagy in the germ cells. Meanwhile, prolonged exposure time increases apoptosis as well as autophagy levels of germ cells in mouse testis. In vitro experiments corroborated the induction of autophagy in spermatocyte by heart stress. Functionally, autophagy functions as an apoptotic inducer rather than a self-protective mechanism in germ cells, because the inhibition of autophagy markedly reduces the apoptotic rate of germ cells in the testis [73].

In addition to triggering cellular dysfunction, autophagy also functions as a cytoprotective response in germ cells under stressful conditions. When treating GC-2 cell with dibutyl phthalate (DPB) significantly induces ER stress in GC-2 cells. However, the expression of caspase-12 or the phosphorylation of JNK or p38 is not changed at the indicated DBP doses, and the inhibition of ER stress increases DBP-induced GC-2 cell apoptosis. Autophagy is participated in the regulation of ER stress, because the inhibition of autophagy significantly aggravated apoptosis. In vivo study indicates that autophagy is consistently induced in rat testis under DBP exposure. The suppression of ER stress or autophagy aggravates DBP-induced injury in rat testis, as evidenced by the greater reduction in testicular index and decrease in germ cells in the seminiferous tubules [74]. Except for exempting germ cells from toxicant insults, autophagy also plays vital roles in germ cells under the exposure of some physical factors. Studies had demonstrated that exposure of spermatocytes to radiofrequency (RF) can lead to the accumulation of intracellular ROS and thereafter inducing autophagy through the activation of ERK signaling pathway. However, the activation of autophagy can dwindle the accumulation of ROS within spermatocytes. Therefore, the induction of autophagy is an indispensable mechanism for germ cell survival [75].

4.3. Functions of autophagy in testicular somatic cell impairment

In addition to the involvement of autophagy in germ cells, studies also evaluated the role of autophagy in testicular somatic cell under toxicant exposure. It had been revealed that exposure of rat Leydig cells to zearalenone (ZEA) leads to the induction of autophagy in Leydig cell. The induction of autophagy is related to the concentration of ZEA, as the expression of LC3-II is peaked at 5 μg/mL and then gradually decreased. In Leydig cells, the activation of autophagy acts as a cytoprotective role in ZEA-treated Leydig cells. Inhibition of autophagy markedly increases the apoptosis level of Leydig cells compared with that of ZEA treatment alone. By contrast, the apoptosis level decreased after the cotreatment of ZEA and rapamycin [76].

Sertoli cells (SCs) orchestrate the processes of spermatogenesis by nourishing and adapting environment for germ cell survival and differentiation. Toxicant-induced dysregulation

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of SCs leads to the reduction in its supportive capacity, thus impairing spermatogenesis and fertility. Studies had revealed that exposure of SCs to 4-Nonylphenol (NP) leads to the upregulation of ROS level, which in turn activates JNK signaling pathway and mediates the induction of JNK-dependent autophagy [77]. Functionally, autophagy acts as a self-protective machinery in SCs under NP treatment, because the inhibition of autophagy considerably increases the level of cell death [78]. Obviously, the abnormal induction of autophagy in SCs under toxicant exposure is also related to the process of apoptosis. For example, see Ref. [79].

5. Concluding remarks

The available data suggest that autophagy is deeply involved in the regulation of testicular homeostasis. For example, autophagy is exerted in the regulation of germ cell survival, the transformation of spermatids, the rearrangement of Sertoli cells, and the testosterone production of Leydig cells (**Table 1**). In mammal, the orchestrated cooperation of germ cells and somatic cells is required for the production of functional sperm. During spermatogenesis, the induction of autophagy is an indispensable mechanism for the paralleled structural transformation of spermatids and Sertoli cells, ensuring that acrosome and flagellum could be successively established. However, it is necessary to illuminate the regulatory roles of hormones such as testosterone, luteinizing hormone (LH), and FSH on the function of autophagy in germ cells under physiological and pathological conditions. It is noteworthy that the decline in fertility result from environmental exposure has caught the worldwide attention recently. Evidences had uncovered that the exposure of testis or cultured testicular cells to adverse environments prompted the initiation of autophagy in both germ cells and somatic cells. However, there is short of relevant data about the regulatory role of autophagy in testicular-related diseases. Most of relevant data are toxicant exposure related, which could not accurately reflect the involvements of autophagy in pathological conditions. It is then the topic of what the optima strategy would be to utilize autophagy to remove deleterious side effects whereupon bring benefits to the therapy of infertility and many other testis related disease.

Cell types	Roles of autophagy	Related dysfunctions
Diploid germ cells	Function as an adaptive response	Germ cell death
Spermatid	Regulation of acrosome and flagellum formation	Sperm head malformation; reduction of sperm motility
Leydig cell	Maintenance of testosterone production	Reduction in testosterone aging
Sertoli cell	Regulation of the formation of ectoplasmic specialization Regulation of tubulobulbar complexes distribution Regulation of androgen-binding protein half-life	Dysregulation of apical ES basal ES perturbation Disorder of Sertoli cell cytoskeleton structure Prolonged ABP half-life

Table 1. Physiological and pathological roles of autophagy in the mammalian testis.

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